

## Protein fraction characterization of sheep milk from the Comisana breed

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### Abbreviations

DTT dithiothreitol

SDS-PAGE sodium dodecyl sulphate polyacrilamide gel electrophoresis

### Introduction

Sheep milk production and its transformation into high quality cheeses with specific characteristics is very important in Italy since there are up to 10 million bred ovine. Among cheeses produced in Italy with ovine milk, there are six productions characterized by guaranteed origin (European Protected Origin) and there is a number of other productions that are still not protected by European marks but that are very much appreciated by Italian customers and also by tourists.

Protein fraction characterization of sheep milk from the Apennine Mountains may be useful to better characterize and typify the raw material “milk” and to transform it into high quality ovine cheese linked to the Country of origin, in order to be protected by a national quality trademark.

Biochemical analyses on protein fractions were performed on sheep milk samples of the Comisana breed reared on a farm of the Marche Region in the Apennine Mountains. In

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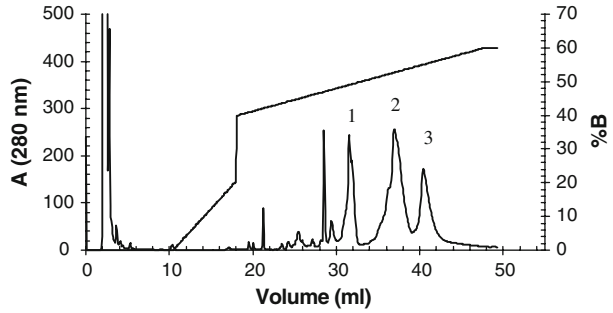
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**Fig. 1** RP-HPLC of skimmed sheep milk. Peak 1,  $\alpha$ -lactalbumin; peaks 2 and 3 are the two variants of  $\beta$ -lactoglobulin

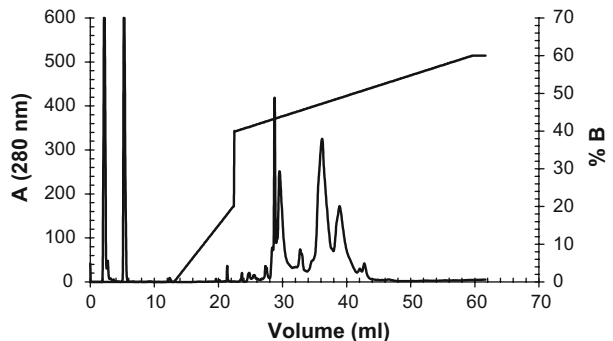


particular, the casein and whey protein fractions were partly purified and quantified in order to give further insight into the technological quality of sheep milk.

### Materials and methods

This study was performed using bulk milk from 94 sheep belonging to the Comisana breed. Milk samples were transported to the laboratory refrigerated at + 4°C, then frozen and kept at – 20°C until used. The chemical analyses were focused on the evaluation of the total casein content, total whey protein content and isolation of the single protein fraction. Skimmed milk was prepared by centrifugation at 3000 g for 30 min at 15°C. Whole casein was obtained from skim milk by adjusting the pH to 4.6 with 1 M HCl and centrifuging at 15000 g for 20 min in order to obtain a supernatant of whey proteins and the isoelectrically precipitated caseins. 100  $\mu$ l of the supernatant was subjected to reversed-phase chromatography on HPLC (RP-HPLC) by using a C4 column, Prosphere 300Å (5  $\mu$ m, 4,6 mm I.D., 150 mm, Alltech) treated by a previously described procedure (Vincenzetti et al. 2006). Whole precipitated caseins were resuspended in buffer A (0.1 M bis-tris pH 8.0; 8.0 M urea; 1.3% trisodic citrate; 0.3% DTT) and subjected to a RP-HPLC performed under the previous conditions. All the proteins separated by the chromatography were analyzed by electrophoresis (13% or 15% SDS-PAGE).

**Fig. 2** RP-HPLC of caseins from skimmed sheep milk



## Results

The total whey protein content of sheep bulk milk was 17.0 g/l, whereas the total casein content was 39.0 g/l. These data confirmed the principal cheese making attributes/characteristics of sheep milk. Sheep milk whey protein characterization by RP-HPLC gave a  $\alpha$ -lactalbumin content (peak 1) of 1.6 g/l, whereas  $\beta$ -lactoglobulin was separated into two variants corresponding to peak 2 (content about 16.7 g/l) and peak 3 (content about 11.0 g/l), as shown in Fig. 1. Sheep milk caseins were separated by RP-HPLC and the result, shown in Fig. 2, indicated a partial separation of the casein fraction.

## Discussion

The results obtained in this study confirmed the specific attributes/characteristics of Comisana breed sheep milk to cheese making evidenced by its high total caseins content as also shown by Chiofalo et al. (2004). The presence of two variants of  $\beta$ -lactoglobulin is interesting as this may influence the technological properties of dairy products (Farrel et al. 2004). The  $\alpha$ -lactalbumin content in sheep milk is higher than that of bovine milk (Zhang and Brew 2003). Since RP-HPLC did not give an accurate separation and individuation of the different casein variants, further proteomic studies are still in progress.

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